## REMARKS

Claims 1-24 are pending and were rejected under § 112, first paragraph as discussed below.

Claims 1 and 24 are being amended to bring the them into compliance with the enablement requirement, in line with the Office's comments set forth in the action (albeit not exactly in the way Examiner Staples initially proposed) and in the Office's interview summary record. The discussion appears in the appropriate section below. Amendments to claim 1 and 24 are identical so will be discussed with reference to claim 1 for simplicity.

Claims 3 and 6 are amended for clarity by insertion of two commas to set off a clause.

New claims 25-28 are added, and are discussed below.

None of the claims introduce new matter and are of appropriate scope to be entered after final rejection, as they do not raise new issues for consideration by the Office and advance the case to allowance. Hence, their entry is respectfully requested.

Applicants thank Examiners Staples and Horlick for their time and graciousness in a lengthy multiparty telephonic interview conducted on April 29, 2009 in which the Office's view of the "missing elements" in claim 1 (and 24) to make these claims "whole" and thereby enabling was discussed thoroughly as were proposals by Applicants to amend the claims to overcome the alleged deficiencies.

From the interview, Applicants were led to understand that the Examiner will contact the undersigned after consideration of the present paper to discuss any issues that may remain for allowance. For example, Applicants understand that the addition of several new claims here will not be considered reflexively or as a *per se* reason for concluding that new issues have been raised. Rather, Applicants understand that any remaining "problems" will be dealt with simply by telephone and, if necessary by an Examiner's amendment rather than by yet another Advisory Action. Applicants thank the Examiner for this consideration.

### I. *WITHDRAWN REJECTIONS*

Applicants thank the Examiner for withdrawing the prior rejections under §112, 2nd paragraph, directed to:

(a) omitted essential structural cooperative relationships of elements with respect to fluorescently labeled probes of sequences NucSeqI, NucSeqII, NucSeqI', and/or NucSeqII', and

(b) omitted essential steps amounting to a gap between the steps.

## II. *NEW CLAIM OBJECTIONS NECESSITATED BY PRIOR AMENDMENT*

Claim 1 was objected to because of what the Examiner correctly noted was a typographical error regarding a repeat in line 4 of NucSeqI when NucSeqI'_ was intended. This error has been corrected by amendment.

Claim 2 was objected to because of the following informality. A ":" had been added and indicated by underlining. However, the claim identifier was incorrectly given as "previously presented" when "currently amended" would have been the proper identifier. However, that informality is now moot as claim 2 has "progressed" to the status of "previously presented" and that underscoring no longer appears, thereby rendering this objection moot.

## III. *REJECTION UNDER 35 USC § 112, First Paragraph (Maintained)*

### A. The Rejection

The rejection of claims 1-24 for lack of enablement was maintained. The Office did not accept Applicants' central argument that in view of the amendments made at the time, claims 1 and 24 (and therefore, the dependent claims) were fully enabled. The amendment to introduce the recitation of quencher and fluorophore did overcome part, but not all of, the pending rejection.

The Office continued to rebut Applicants' contention that the specification enabled the claims as written with respect to being able to determine copy number from weights or concentrations (wt/vol). Claims 1 and 24 were alleged by the Office to lack an essential element that would permit linking of weights or concentrations (*e.g.*, wt/vol) to copy number). The Examiner therefore found the equation for determining Relative CN to be in error because it "left out" such required information.

The various concepts, the equation, methods of carrying out the invention, and the meaning of the terms were discussed extensively during the telephone interview. The Examiner reminded applicants (in the interview and Summary) that essential steps and elements of the claimed invention should be recited, especially with regard to relative CN of the unknown sequences being determined from the two standards curves constructed from known w/v concentrations of the standards wherein the unknown sequences could vary in composition and/or length (and hence, MW) , from those standards.

**B. Applicants' Response**

In response, Applicants have amended claims 1 and 24 as discussed below, and believe that all the necessary elements for compliance with § 112, first paragraph, are now present.

The opening clause of part (2) of claim 1 has been amended for simplicity and clarity.

The language of steps (i) –(iv) in claim 1 (below step (d)) has been amended by condensing to 3 steps (now (i) – (iii) for greater clarity. First the statement that NucSeqI' and NucSeqII' are both localized on a single vector is combined with the statement that the ratio of NucSeqI' to NucSeqII' is known. Note also that the term "concentration" has been removed because it is the ratio of the two sequences (not necessarily the ratio of their concentrations) that must be known. Indeed a key contribution of the present invention to the art is the greater accuracy in determining copy number provided by the presence of NucSeqI' and NucSeqII' on one vector (part (2)(i) of the claim.

Part (3) of prior claim 1 has been expanded to three parts (now (3)-(5)) for greater clarity and to better indicate how the process steps include all the requisite elements so that the formula appearing in part (5) is proper, and the determination of relative CN is performed in compliance with § 112, first paragraph.

Part (3) now recites:

*"(3) determining the results of the amplifications of step (2) expressed as threshold cycle (Ct);"*

as disclosed in the specification (see Examples and Drawings). As used in the specification, and as is well-known in the art, Ct is the point at which the fluorescence crosses the threshold. For example, a detailed 'primer' on this technology, discussing this term is found at (http://pathmicro.med.sc.edu/pcr/realtime-home.htm). The relevant section is reproduced below as Appendix A hereto, along with Appendix B which is a page at the Applied Biosystems website termed "Real-Time PCR: Understanding $C_T$) at the URL.
http://www3.appliedbiosystems.com/cms/groups/mcb_marketing/documents/generaldocuments/cms_053906.pdf.

Step 4 now separately recites obtaining concentration or quantity of NucSeqI and NucSeqII from the respective standard curves, as follows:

*"(4) obtaining from the results in step (3) the following values:*

*(i) "Conc-$I_{SCI}$" which is the **concentration or quantity** in the sample of NucSeqI determined from standard curve $SC_I$; and*

*(ii) "Conc-$II_{SCII}$" which is the **concentration or quantity** in the sample of NucSeqII determined from standard curve $SC_{II}$ which standard curves express threshold cycle as a function of said concentration or quantity; and"*

Step (5) now recites separately the determination of the relative CN of the unknown sequences from the **concentration or quantity** of NucSeqI and NucSeqII obtained in step (4):

*(5) determining from the values obtained in step (4) the relative CN of NucSeqI with respect to NucSeqII by the formula:*

$$\text{Relative CN} = \frac{\text{Conc-}I_{SCI}}{\text{Conc-}II_{SCII}}"$$

Note that in Step 4, the term *"Conc"* as it relates to NucSeqI and NucSeqII is altered in its definition to be either a formal concentration term or a quantity term, as the units are "canceled" mathematically in step (5) to yield a unitless ratio.

Applicants have thus amended the concentration terms in the formula of claims 1 and 24 to be "concentration" or "quantity" in the sample. Quantity can also be viewed as copy number in the sample. (Applicants believe this to be understood without a need for its explicit recitation in the claims, though it is added in new claims 25 and 27). The foregoing is directly supported in the Examples section of the specification, particularly in the drawings, Figures 1-4, in which the starting quantities, as copy numbers, are shown on the (logarithmic) X axis. Applicants emphasize that because in the end, a ratio (relative copy number) is determined, the results would be the same if the "Conc" values in the formula referred to actual concentrations (whether w/v or molar), amounts, dilutions, *etc.*

Applicants reiterate that their invention is contributes the following new elements to the art. As a "reference vector," a single reference nucleic acid is used which includes **all** reference sequences (*i.e.*, NucSeqI' and NucSeqII'… *or more if desired*) in a known (predetermined) ratio, that can be, for example, 1:1 (as in the Example provided below). As a result, since the ratio of the "reference" sequences in the "reference" vector is precisely known { recited in part (2)(i) of amended claim 1}, this reference vector can be used for relative quantification (from copy number, or quantity expressed otherwise, or concentration) of two (or more) unknown, queried sequences (*e.g.*, genes or transcripts), to determine how many copies of "gene I" or "transcript I" are present in, *e.g.*, a cell, per copy of "gene II" (or "transcript II).

***Example A*** provided in Applicants' prior response is presented below in "improved" and simpler form to illustrate why the determination set forth in the claims (including the calculations and formula) does not require any information on the (potential difference in) lengths and therefore, of molecular masses or molar concentrations or w/v concentrations of NucSeqI and NucSeqII (or NucSeqI' and NucSeqII'). Several graphs and diagrams are provided to help clarify this Example.

### C. ***REVISED "EXAMPLE A"***

**Task**: To determine relative copy number of viral DNA in an infected cell.

**Sample** used in method: extract of cellular DNA from virus-infected cells.

NucSeqI: Gene I, which is a viral gene X (Vx) – 1 copy per viral genome.

NucSeqII: Cellular gene Y (Cy) – 1 copy per cell genome.

NucSeqI' 100 base pair segment of viral gene Vx serves as unique probe for gene Vx.

NucSeqII 140 base pair segment of cell gene Cy serves as probe for cell gene Cy

**Ultimate determination:** ratio of the number of copies of Vx to Cy in the sample *(which can be related back to "cells", i.e., relative number of viruses per cell)*

"Reference" **vector** of claim 1(2)(i) includes the following

- 1 copy of NucSeqI' and 1 copy of NucSeqII' (so their ratio=1, for simplicity)
  - This ratio could be 2, 3, *etc.*. The key is that this ratio is known

**Procedure:**

(1) Serial dilutions of solution containing "reference vector" are made

(2) NucSeqI-containing sample and NucSeqI'-containing reference vector (which also contains Nuc SeqII') are amplified in one container {claim 1(2)(a) and (c)}.

(3) NucSeqII-containing sample and NucSeqII-containing reference vector (which also contains Nuc SeqI') are amplified in one container {claim 1(2)(b) and (d)}.

Amplification (as RT-PCR) is performed to determine the threshold cycle (Ct). In RT-PCR, the **cycle number at which the increase in fluorescence (and therefore DNA) is exponential** is measured, This is exemplified by the orange horizontal line (known as the threshold) and is set by the user, as shown in general illustrative Fig. A below. The point at which the fluorescence crosses the threshold is called the **Ct**.

(This Figure originates from the website referred to above and in Appendix A: (http://pathmicro.med.sc.edu/pcr/realtime-home.htm)

**Fig. A**

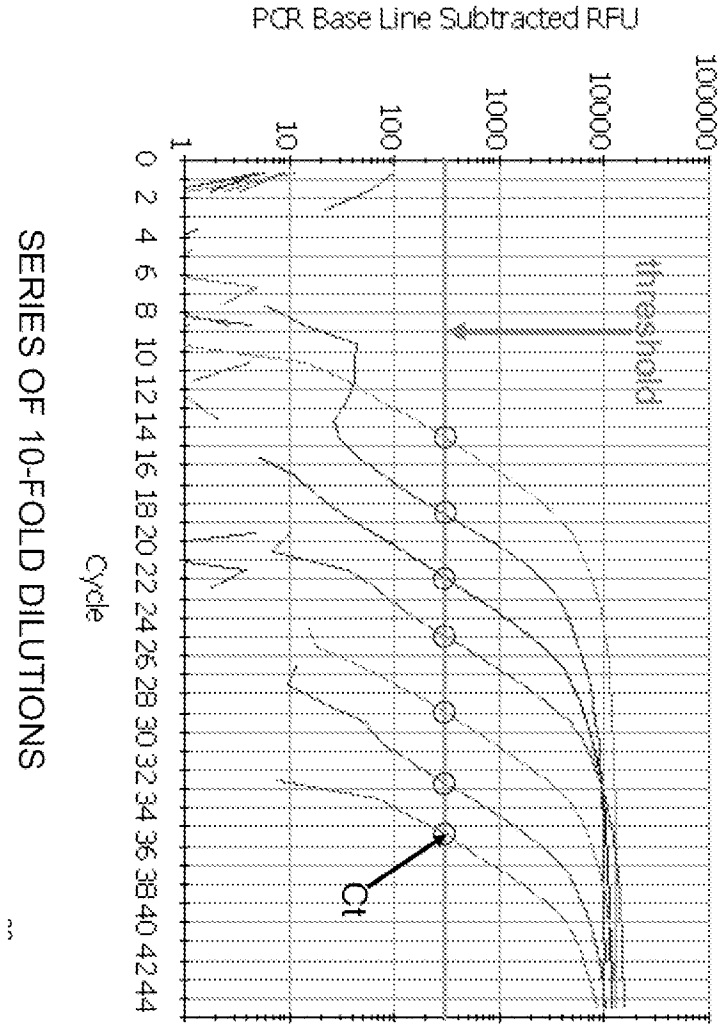



Fig. B, below is another illustrative view available after completion of the run - an amplification window. This window shows the amount of fluorescence obtained in each amplification cycle for each reaction. The threshold cycle ($C_t$) is shown by the darker horizontal line.

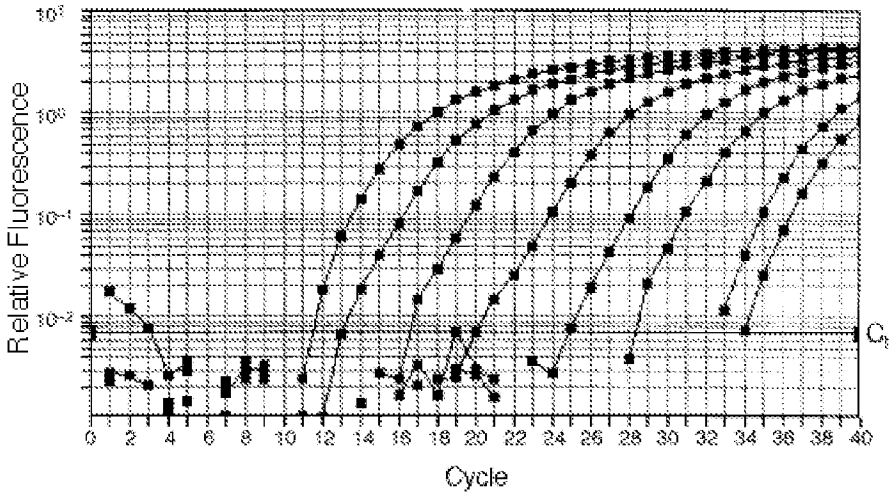



**FIG. B**

Again, Ct is the standard (threshold cycle) measure used routinely in RT-PCR. It is a standard measure of the number of amplification cycle needed to reach a detectable fluorescent signal (in linear range).

To obtain standard curves $SC_I$ and $SC_{II}$ , copy numbers (across dilutions) of NucSeqI' and NucSeqII' (both residing together on a single vector) are plotted against the Ct (threshold cycle) (Fig. C).

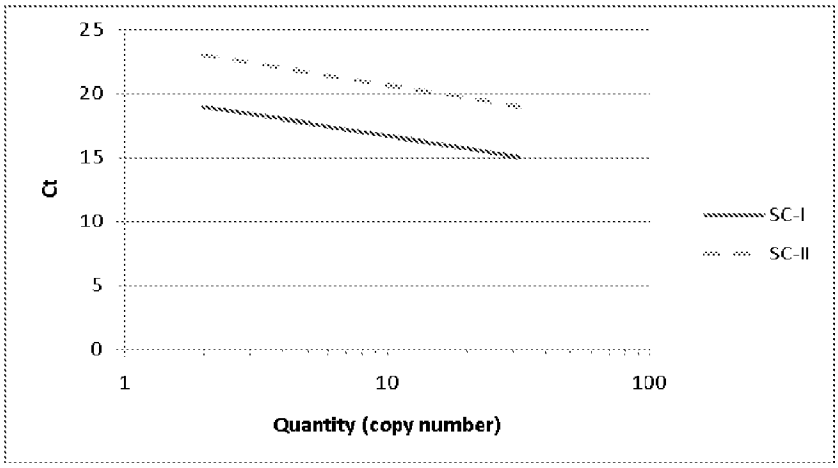


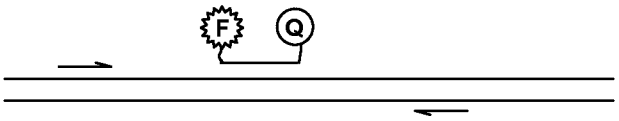

**Fig. C**

By amplifying NucSeqI and NucSeqII, Ct values of NucSeqI and of NucSeqII are obtained.

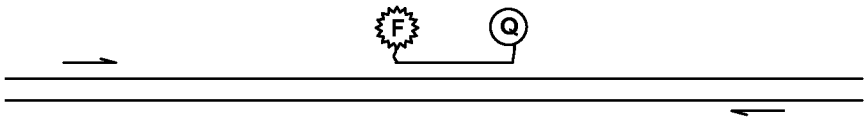
Since one nucleic acid sequence will only bind one probe, and since the number of queried nucleic acid sequences **(NucSeqI vs NucSeqII)** and "reference" sequences (NucSeqI' vs. NucSeqII')) is doubled in each amplification cycle, **any difference in length of the queried nucleic acid sequence or reference sequences does not affect the analysis.** For that reason,

any difference in actual weight (MW, ng/ml, *etc.*) of these nucleic acid sequences does not affect the amount of fluorescence signal that is generated.

Let's take the example of NucSeqI' being 100 bp (F is the fluorophore, Q is the quencher)




and NucSeqII' being 140 bp




Ct's are determined and the corresponding quantity (=copy number) of NucSeqI and NucSeqII are read from the standard curves $SC_I$ and $SC_{II}$ as shown below `in Fig. D:

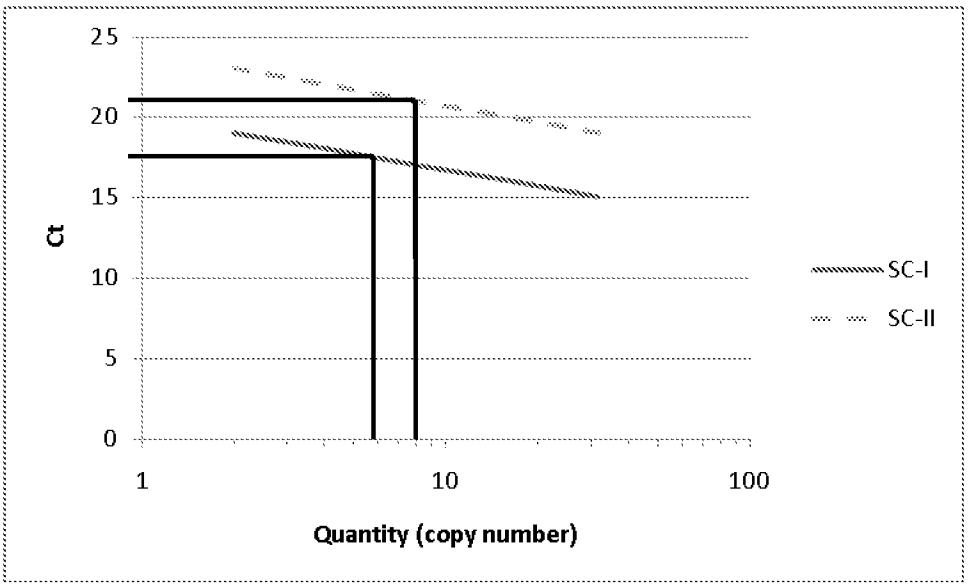



**Fig. D**

For this example, assume that the measured Ct of NucSeqI (which is contained in a "gene I" = Viral gene Vx in the example) corresponds to a NucSeqI' copy number of **6** and the measured Ct of NucSeqII (contained in a "gene II", here cellular gene Cy) corresponds to a NucSeqII' copy number of **8.** As stated above, for this example, we are assuming that the "known" ratio {per claim 1(2)(°i)} of NucSeqI' to NucSeqII' in the **single vector** is 1 (1:1).

Using the amended definition of Conc-$I_{SCI}$ and Conc-$I_{SCII}$, it follows that Conc-$I_{SCI}$ is 6:1 = 6 (copies) , and Conc-$II_{SCI}$ is 8:1 =8 (copies). According to the formula of the claim, the Relative CN = 6/8 = 0.75. Therefore, the sample has 0.75 copies of the NucSeqI (representing viral gene Vx) per copy of NucSeqII (representing cellular gene Cy, ). Said otherwise, there are 75 copies of Vx (containing NucSeqI) per 100 copies of Cy (containing NucSeqII) (or per 100 cells).

Applicants believe that to amend the claims by introducing an element of **relative probe weights** (as suggested by the Examiner) would not only be unduly restrictive, but, in fact, would be unnecessary, since, as discussed above, the relative probe weights or probe lengths bear no relevance to the calculated outcome. Note that the claims *do* imposed a limit on the lengths of NucSeqI relative to NucSeqI' (and a similar limit on the lengths of NucSeqII relative to NucSeqII'). See claim 1(c) and 1(d).

Again, support for the amendments to the claims are found in the specification, page 1, lines 23-34, and more importantly, in the X axes of Figures 1-4, which shows the values as "starting quantity" (= "copy number").

Applicants re-emphasize that, NucSeqI' and NucSeqII' are localized on a single vector {See: claim 1(2)(i)} and the relative molar concentration of NucSeqI' and NucSeqII' is an inherent, and known feature of the vector as constructed. (In the present Example A above, each vector molecule has one copy of NucSeqI' and one copy of NucSeqII').

Moreover, as is the usual practice in the art for maximum reliability of results, these sequences would be amplified "together" with the unknown/queried sequences (NucSeqI and NucSeqII). Thus, the skilled artisan would perform the amplifications simultaneously with NucSeqI and NucSeqII in one 'tube' and NucSeqI' and NucSeqII' in one 'tube.' They would be subject to the same "reaction environment," use the same "master mix" of ingredients, *etc.* Again, these features are not specified in the claims as they are part of what is accepted as "routine" in this art.

It should be noted again that present method has the distinct advantage that wt/vol or molar concentration or the absolute or relative lengths of the sequences being amplified (*e.g.*, NucSeqI' and II') are not relevant to the calculated outcome.

New dependent claims 25-28 (which depend from claim 1 or 24) specify which quantity or concentration (or relative dilution) is actually plotted in the standard curve in order to obtain the concentration or quantity of the unknown sequences. Based on the telephonic interview,

Applicants were left with the impression that the Examiner did not object to use of the terms "concentration" or "quantity" in claim 1 (and 24) based on Applicants' further explanation of the invention. Therefore, Applicants believe that these new claims are proper, do not raise new issues, and should be allowable as well.

Applicants believe that the foregoing amendments to claim 1 (and identical amendments to claim 24) have clarified the remaining issues raised by the Examiner in his enablement rejection and show, with the aid of the discussion above, how the claims now recite every requisite step, concept or value needed to practice the method. For these reasons, it would be proper to withdraw the enablement rejection.

## IV. CONCLUSION

In view of the amendments to the claims and the foregoing remarks, Applicants believe that they have overcome or mooted the remaining objections and rejection. Reconsideration, withdrawal of the rejections and allowance of the amended claims are respectfully requested.

**As discussed in the interview, the Examiner is respectfully requested to contact the undersigned at (202) 628-5197 to discuss any further minor amendments, *etc.*, that may be needed prior to allowance.**

Dated: May 26, 2009

Respectfully submitted,

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